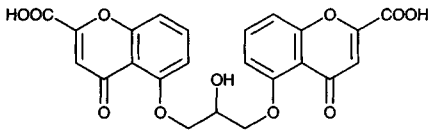
Cromolyn



Molecular formula: $C_{23}H_{16}O_{11}$, $C_{23}H_{14}Na_2O_{11}$ (disodium salt)
Molecular weight: 468.37
CAS Registry No.: 16110-51-3, 15826-37-6 (disodium salt)
Merck Index: 2658
Lednicer No.: 3 66, 235; 4 44, 137, 150, 205

SAMPLE

Matrix: blood, microsomal incubations, tissue
Sample preparation: Microsomal incubation. Add 200 μL microsomal incubation to 800 μL MeCN:DMSO:MeOH 60:5:35, vortex for 20 s, centrifuge. Dilute the supernatant two-fold with water, inject a 20 μL aliquot. Tissue. Homogenize tissue with 3 volumes MeCN:DMSO:MeOH 60:5:35, centrifuge, dilute with water, inject an aliquot.

HPLC VARIABLES

Guard column: Hypersil ODS-2 C8
Column: 100 × 4.0 Hypersil ODS-2 C8
Mobile phase: MeOH:4mM benzyltributylammonium chloride 48.5:51.5
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 3.95
Limit of detection: ⩽ 30 ng/mol

KEY WORDS

lung; liver; kidney; human; dog; rat; rabbit; pharmacokinetics

REFERENCE

Saah,F.; Wu,W.-M.; Eberst,K.; Marvanyos,E.; Bodor,N. Design, synthesis, and pharmacokinetic evaluation of a chemical delivery system for drug targeting to lung tissue, *J.Pharm.Sci.*, **1996**, *85*, 496–504.

SAMPLE

Matrix: urine
Sample preparation: Dilute urine 10-fold with water, filter (Chromatodisc 25A, Kurabou, Osaka), inject a 50-200 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Capcell Pak C18 SG-120 (Shiseido)
Mobile phase: MeOH:35 mM pH 8 phosphate buffer 30:70, containing 75 mM hydrogen peroxide and 20 mM 18-crown-6
Flow rate: 0.6
Injection volume: 50-200
Detector: F ex 325 em 448 following post-column reaction. The column effluent flowed through a 3 m × 0.25 mm ID Tefzel coil irradiated by two 4 W germicidal lamps. The effluent from this coil flowed through a 50 cm × 0.13 mm ID PEEK coil and a 2 m × 0.25 mm ID PTFE coil to the detector.

CHROMATOGRAM

Retention time: 18
Limit of detection: 400 ng/mL

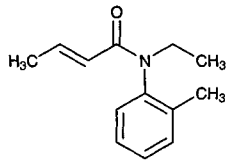
KEY WORDS

post-column reaction; post-column photochemical derivatization

REFERENCE

Mawatari,K.-i.; Mashiko,S.; Sate,Y.; Usui,Y.; Iinuma,F.; Watanabe,M. Determination of disodium cromoglycate in human urine by high-performance liquid chromatography with post-column photoirradiation-fluorescence detection, *Analyst*, **1997**, *122*, 715–717.

Crotamiton



Molecular formula: $C_{13}H_{17}NO$
Molecular weight: 203.28
CAS Registry No.: 483-63-6
Merck Index: 2661

SAMPLE

Matrix: blood, urine
Sample preparation: Evaporate 40 µL 61 µM IS in MeOH into a tube with a stream of nitrogen, add 1 mL plasma or 150 µL urine, add 1 mL pH 12 buffer (Titrisol, Merck), add 4 mL n-heptane:isoamyl alcohol 99:1, shake for 20 min at 300 rpm, centrifuge at 2500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 µL mobile phase, inject a 75 (plasma) or 65 (urine) µL aliquot.

HPLC VARIABLES

Column: 200 × 4.6 5 µm Hypersil ODS
Mobile phase: MeCN:10 mM KH_2PO_4 45:55
Flow rate: 1.5
Injection volume: 65-75
Detector: UV 220

CHROMATOGRAM

Retention time: 6 (trans), 7 (cis)
Internal standard: N-ethyl-N-propionyl-o-toluidine (5)
Limit of quantitation: 320 nM (urine), 43.3 nM (plasma)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Sioufi,A.; Sandrenan,N.; Dubois,J.P. Determination of crotamiton in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *494*, 361–367.

SAMPLE

Matrix: formulations
Sample preparation: Dissolve in THF, inject a 5 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 3 µm Spherisorb CN
Column: 250 × 2 3 µm Spherisorb CN
Mobile phase: carbon dioxide and MeOH
Column temperature: 100
Flow rate: 1.9 (carbon dioxide), 0.05 (MeOH)
Injection volume: 5
Detector: UV 210

CHROMATOGRAM

Retention time: 1.9 (cis), 3.3 (trans)

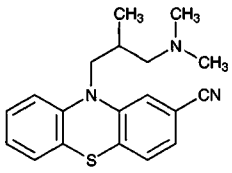
OTHER SUBSTANCES

Simultaneous: impurities, excipients

KEY WORDS
cream; SFC; 125 bar

REFERENCE
Anton,K.; Bach,M.; Geiser,A. Supercritical fluid chromatography in the routine stability control of antipruritic preparations, *J.Chromatogr.*, **1991**, *553*, 71–79.

Cyamemazine



Molecular formula: $C_{19}H_{21}N_3S$
Molecular weight: 323.46
CAS Registry No.: 3546-03-0
Merck Index: 2753

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 269

CHROMATOGRAM

Retention time: 6.71

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 270

CHROMATOGRAM

Retention time: 14.993

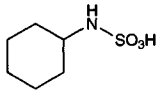
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cyclamic acid



Molecular formula: $C_6H_{13}NO_3S$
Molecular weight: 179.24
CAS Registry No.: 100-88-9, 139-05-9 (sodium salt), 139-06-0 (calcium salt)
Merck Index: 2770

SAMPLE

Matrix: food

Sample preparation: Fruit juice. Centrifuge at 3000 g for 20 min, filter (0.45 μm) the supernatant. Remove a 2 mL aliquot of the filtrate and add it to 1 mL 30% hydrogen peroxide and 300 μL 37% HCl, heat at 100° under a reflux condenser for 1 h, cool to room temperature, neutralize with 400 μL 40% NaOH, make up to 25 mL with pH 10 borate buffer. Remove a 1 mL aliquot and make up to 10 mL with reagent, after 5 min inject an aliquot. Marmalade, preserves. Stir 10 g marmalade or preserve with 50 mL water for 30 min, centrifuge at 3000 g for 20 min, remove the supernatant, suspend the residue in 10 mL water, centrifuge. Combine the supernatants and make up to 100 mL with water. Remove a 2 mL aliquot and add it to 1 mL 30% hydrogen peroxide and 300 μL 37% HCl, heat at 100° under a reflux condenser for 1 h, cool to room temperature, neutralize with 400 μL 40% NaOH, make up to 25 mL with pH 10 borate buffer. Remove a 1 mL aliquot and make up to 10 mL with reagent, after 5 min inject an aliquot. (Prepare borate buffer by dissolving 25 g boric acid in 900 mL water, adjust pH to 10 with KOH. Prepare reagent by dissolving 200 mg o-phthaldialdehyde in 5 mL EtOH, add 1 mL 3-mercaptopropionic acid, make up to 100 mL with borate buffer.)

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrospher 100 RP-18
Mobile phase: MeCN:buffer 64:36 (Prepare buffer by dissolving 3 g $Na_2HPO_4.12H_2O$ and 3 g $NaH_2PO_4.H_2O$ in 1 L water.)
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 350 em 440-650

CHROMATOGRAM

Retention time: 12
Limit of detection: 500 ng/g

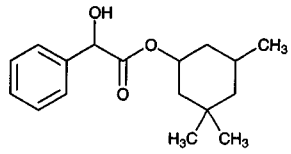
KEY WORDS

derivatization; fruit juice; marmalade; preserves

REFERENCE

Rüter,J.; Raczek,D.I.U. Empfindliches und selektives HPLC-Verfahren mit prächromatographischer Derivatisierung zur Bestimmung von Cyclamat in Lebensmitteln [Sensitive and selective HPLC procedure with prechromatographical derivatization for the determination of cyclamate in foods], *Z.Lebensm.Unters.Forsch.*, **1992**, *194*, 520–523.

Cyclandelate



Molecular formula: $C_{17}H_{24}O_3$
Molecular weight: 276.38
CAS Registry No.: 456-59-7
Merck Index: 2771
Lednicer No.: 1 94

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 5 mL 100 mM NaOH + 10 mL chloroform, shake mechanically for 10 min, centrifuge at 600 g for 10 min. Remove the aqueous layer and add it to 1 mL 2 M HCl, shake with 10 mL diethyl ether, centrifuge at 600 g for 10 min. Remove 8 mL of the organic layer and add it to 4 mL 10 mM NaOH, shake for 15 min, centrifuge at 600 g for 10 min. Remove 4 mL of the aqueous layer and add it to 500 µL 1 M HCl, add 2 mL reagent, add 300 µL hexane, shake, centrifuge at 600 g for 10 min, inject a 100 µL aliquot of the hexane layer. (Reagent was 36.8 g $Ce(NH_4)_4(SO_4)_4.2H_2O$ in 1 L 1 M sulfuric acid, stir for 30 min to dissolve, allow to stand overnight, filter.)

HPLC VARIABLES

Column: 250 × 4.6 5 µm Zorbax CN
Mobile phase: Hexane:propanol 1000:1
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 4.4 (benzaldehyde, oxidation product of the major metabolite, mandelic acid)
Limit of detection: 100 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Kojima,K.; Uezono,Y.; Takahashi,T.; Nakanishi,Y. High-performance liquid chromatographic method for the determination of a cyclandelate metabolite, mandelic acid, in human plasma, *J.Chromatogr.*, **1988**, *425*, 203–207.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 26.383

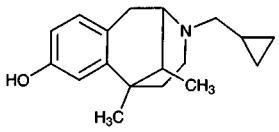
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cyclazocine



Molecular formula: $C_{18}H_{25}NO$
Molecular weight: 271.40
CAS Registry No.: 3572-80-3
Merck Index: 2773
Lednicer No.: 1 298

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.7

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

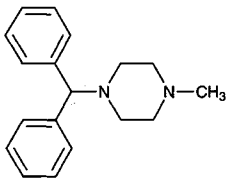
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phe-

nobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Cyclizine



Molecular formula: $C_{18}H_{22}N_2$
Molecular weight: 266.39
CAS Registry No.: 82-92-8, 303-25-3 (HCl), 5897-19-8 (lactate)
Merck Index: 2779
Lednicer No.: 1 58

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 6.49
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 5 mL MeCN and 5 mL water. 1 mL Serum or 500 μL urine + 1 mL MeCN, vortex for 30 s, centrifuge at 1600 g for 5 min. Remove the supernatant and add it to 4 mL water and 100 μL 1 μg/mL IS, vortex for 30 s, add to the SPE cartridge, wash with 20 mL water, wash with 5 mL MeCN:water 70:30, dry under vacuum, elute with three 500 μL aliquots of MeCN:50 mM pH 3 sodium phosphate buffer 70:30. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 20 μL water, vortex for 1 min, add 30 μL MeCN, vortex for 1 min, centrifuge at 1600 g for 30 s, inject a 5-15 μL aliquot of the clean supernatant.

HPLC VARIABLES

Guard column: Uptight Precolumn packed with glass beads (Upchurch)

Column: 150 × 4.1 5 μm Techsil C18 (HPLC Technology)

Mobile phase: MeCN:50 mM pH 3 phosphate buffer 30:70 (Buffer was 3.2 mL/L phosphoric acid in water adjusted to pH 3.0 with solid NaOH.)

Column temperature: 30

Flow rate: 1

Injection volume: 5-15

Detector: E, Environmental Sciences Associates Model 5100A Coulochem, Model 5010 analytical cell preceded by a carbon filter, first electrode 0.55 V, second electrode 0.90 V, Model 5020 guard cell preceded by a carbon filter +1.00 V

CHROMATOGRAM

Retention time: 6

Internal standard: chlorcyclizine (12)

Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; SPE

REFERENCE

Walker,R.B.; Kanfer,I. Sensitive high-performance liquid chromatographic determination of cyclizine and its demethylated metabolite, norcyclizine, in biological fluids using coulometric detection, *J.Chromatogr.B*, **1995**, *672*, 172–177.

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 50 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 50 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.3

OTHER SUBSTANCES

Also analyzed: chlorpheniramine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS

tablets; syrups; elixirs; injections

REFERENCE

Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE

Matrix: formulations

Sample preparation: Capsules, tablets. Remove contents of capsules and powder tablets. Weigh out an amount equivalent to about 10 mg loperamide hydrochloride, add 80 mL chloroform, shake for 15 min, make up to 100 mL with chloroform, filter and discard first 10-20 mL of filtrate. 5 mL Filtrate + 1 mL 400 μg/mL cyclizine hydrochloride in chloroform, make up to 25 mL with chloroform, inject an aliquot. Syrups. Add a quantity of syrup corresponding to about 10 mg loperamide hydrochloride to 30 mL water, extract four times with 20 mL portions of chloroform and filter each extract through glass wool, combine the extracts and make up to 100 mL with chloroform. Remove a 5 mL aliquot and add it to 1 mL 400 μg/mL cyclizine hydrochloride in chloroform, make up to 25 mL with chloroform, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Perkin-Elmer Analytical silica
Mobile phase: Chloroform:MeOH:ammonia 95.5:4.5:0.05
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: cyclizine

OTHER SUBSTANCES

Simultaneous: loperamide
Noninterfering: propylene glycol

KEY WORDS

capsules; tablets; syrups; normal phase; cyclizine is IS

REFERENCE

Leung,C.P.; Au-Yeung,C.Y. High-performance liquid chromatographic determination of loperamide hydrochloride in pharmaceutical preparations, *J.Chromatogr.*, **1988**, *449*, 341–344.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thiorida-

zine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 2.47

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18

Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min

Flow rate: 1.5

Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, tripelennamine, triprolidine, methaphenilene, pyrrobutamine, meclizine, buclizine

REFERENCE

Vydac HPLC Catalog, 1994-5,

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 12.40 (A), 5.78 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

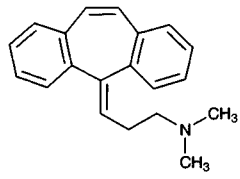
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Cyclobenzaprine



Molecular formula: $C_{20}H_{21}N$
Molecular weight: 275.39
CAS Registry No.: 303-53-7, 6202-23-9 (HCl)
Merck Index: 2782
Lednicer No.: 3 77

SAMPLE

Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL ice-cold MeOH, vortex, centrifuge at 14000 rpm for 10 min, remove the supernatant, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:0.085% phosphoric acid adjusted to pH 6.5 with triethylamine 60:40
Flow rate: 1.5
Injection volume: 80
Detector: UV 229

CHROMATOGRAM

Retention time: 18.4

OTHER SUBSTANCES

Extracted: metabolites

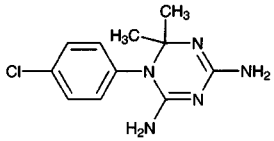
KEY WORDS

liver

REFERENCE

Wang,R.W.; Liu,L.; Cheng,H. Identification of human liver cytochrome P450 isoforms involved in the in vitro metabolism of cyclobenzaprine, *Drug Metab.Dispos.*, **1996**, *24*, 786–791.

Cycloguanil



Molecular formula: $C_{11}H_{14}ClN_5$
Molecular weight: 251.72
CAS Registry No.: 516-21-2, 609-78-9 (pamoate)
Merck Index: 2790
Lednicer No.: 1 281

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 243

CHROMATOGRAM

Retention time: 4.19
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 10.793

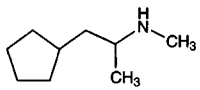
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cyclopentamine



Molecular formula: $C_9H_{19}N$
Molecular weight: 141.26
CAS Registry No.: 102-45-4, 3459-06-1 (HCl)
Merck Index: 2808

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

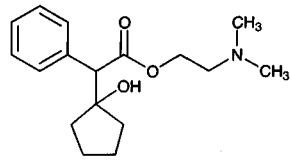
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ran-

itidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Cyclopentolate



Molecular formula: $C_{17}H_{25}NO3$
Molecular weight: 291.39
CAS Registry No.: 512-15-2, 5870-29-1 (HCl)
Merck Index: 2815
Lednicer No.: 1 92

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 75:20:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 260

KEY WORDS
chiral; α = 1.13 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

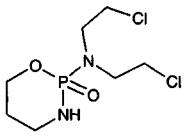
HPLC VARIABLES
Column: 150 × 2 PRP-1 (Keystone)
Mobile phase: MeCN:2-butanone:100 mM pH 7.5 phosphate buffer 40:20:40
Flow rate: 0.15
Injection volume: 1
Detector: chemiluminescence following post-column reaction. A 1 mM solution of Ru(2,2'-bipyridine)$_3^{++}$ in 50 mM sodium sulfate (continuously sparged with helium) was oxidized to Ru(2,2'-bipyridine)$_3^{+++}$ using a Princeton Applied Research Model 174A polarographic analyzer with a platinum gauze working electrode, a platinum wire auxiliary electrode, and a silver wire reference electrode. The Ru solution at 0.3 mL/min was mixed with the column effluent in the flow cell of the detector, a fluorescence detector with the light source removed.

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.1-1 μg/mL

OTHER SUBSTANCES
Simultaneous: atropine, cyclobenzaprine, dicyclomine, procyclidine

REFERENCE
Holeman,J.A.; Danielson,N.D. Microbore liquid chromatography of tertiary amine anticholinergic pharmaceuticals with tris(2,2'-bipyridine)ruthenium(III) chemiluminescence detection, *J.Chromatogr.Sci.*, **1995**, *33*, 297–302.

Cyclophosphamide



Molecular formula: $C_7H_{15}Cl_2N_2O_2P$
Molecular weight: 261.09
CAS Registry No.: 50-18-0, 6055-19-2 (monohydrate)
Merck Index: 2816
Lednicer No.: 3 161

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak SPE cartridge with 10 mL MeOH, 10 mL air, and 10 mL water. 1 mL Serum + 15 μg IS + 5 mL water, mix, pass through the SPE cartridge, wash with 20 mL water, pass 10 mL air through the cartridge, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute with 100 μL water, vortex for 1 min. Add 40 μL toluene, vortex for 30 s, centrifuge at 12000 g for 1 min. Remove a 75 μL aliquot of the aqueous phase and add it to 2.5 mL chloroform, vortex for 1 min, centrifuge at 2000 g for 2 min. Remove a 2 mL aliquot of the chloroform layer, dry under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water, vortex for 1 min, inject a 90 μL aliquot. (Caution: Chloroform is a carcinogen !)

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18, Temp CT 38
Mobile phase: MeCN:2 mM pH 4.0 potassium phosphate buffer 29:71
Flow rate: 1.5
Injection volume: 90
Detector: UV 195

CHROMATOGRAM

Retention time: 7
Internal standard: 5-ethyl-5-p-tolylbarbituric acid (12)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophenon, allopurinol, aspirin, carbamazepine, cisplatin, digoxin, ethosuximide, 5-fluorocytosine, 5-fluorouracil, methotrexate, phenytoin, primidone, theophylline, vincristine
Interfering: carbamazepine-10,11-epoxide, phenobarbital

KEY WORDS

serum; SPE; pharmacokinetics

REFERENCE

Hardy,R.W.; Erlichman,C.; Soldin,S.J. High-performance liquid chromatographic measurement of cyclophosphamide in serum, *Ther.Drug Monit.*, **1984**, *6*, 313–318.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Isolute CH(EC) SPE cartridge with 2 mL MeOH and 1 mL 10 mM ammonium acetate buffer. Mix 500 μL plasma with 1.5 mL 10 mM pH 4.9 ammonium acetate buffer, 50 μL 5 μg/mL IS. Add to the SPE cartridge, wash with 1 mL MeOH:10 mM ammonium acetate 10:90, elute with 300 μL MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm LiChroCART 4-4 RP-select B

Column: 250 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeOH:100 mM pH 4.9 ammonium acetate buffer 60:40
Flow rate: 1
Injection volume: 200
Detector: MS, Finnigan MAT TSQ 700, m/z 261, APCI/ESI interface, vaporizer 450°, capillary 175°, corona discharge 5 μA, argon collision gas 0.5 mtorr, collision energy -45 eV

CHROMATOGRAM
Retention time: 5
Internal standard: deuterated cyclophosphamide (5)
Limit of quantitation: 25 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Fox,P.A.; Lively,J.D.; Firth,J.W.; Woolfrey,S.G.; Greenslade,D. A sensitive assay for cyclophosphamide in human plasma utilizing mass spectrometry, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1047–1059.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg cyclohexyl SPE cartridge (Varian) with two 1 mL portions of MeOH and 1 mL water (pH 4). Add 2 mL serum to 2 mL 25 mM pH 4 phosphate buffer and 400 μL water (pH 4), vortex for 30 s, add four 1 mL portions to the SPE cartridge, let them pass through for 3-4 min, dry the cartridge using full vacuum, wash with 1 mL MeCN:water (pH 4) 10:90, elute with 1 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C8
Mobile phase: MeCN:25 mM pH 4 phosphate buffer 25:75
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Retention time: 7.6

KEY WORDS
pharmacokinetics; serum; SPE

REFERENCE
Corlett,S.A.; Chrystyn,H. High-performance liquid chromatographic determination of the enantiomers of cyclophosphamide in serum, *J.Chromatogr.B*, **1996**, *682*, 337–342.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg cyclohexyl SPE cartridge (Varian) with two 1 mL portions of MeOH and 1 mL water (pH 4). Add 2 mL serum to 2 mL 25 mM pH 4 phosphate buffer and 400 μL water (pH 4), vortex for 30 s, add four 1 mL portions of sample to the SPE cartridge, let them pass through for 3-4 min, dry the cartridge using full vacuum, wash with 1 mL MeCN:water (pH 4) 10:90, elute with 1 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot onto column A, elute with mobile phase, monitor the effluent from column A, divert the effluent from column A containing the cyclophosphamide onto column B, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 4.6 5 μm Spherisorb C1 (packed in house); B 100 × 4.0 Chiral-AGP (Chromtech, Sweden)
Mobile phase: MeCN:15 mM pH 4 phosphate buffer 1:99
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Retention time: 4.54 (R), 8.48 (S)
Limit of detection: 1250 pg/mL

KEY WORDS
chiral; pharmacokinetics; serum; SPE; column-switching; heart cut

REFERENCE
Corlett,S.A.; Chrystyn,H. High-performance liquid chromatographic determination of the enantiomers of cyclophosphamide in serum, *J.Chromatogr.B*, **1996**, *682*, 337–342.

SAMPLE
Matrix: formulations
Sample preparation: Filter (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere reverse-phase
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 2.75

OTHER SUBSTANCES
Simultaneous: ondansetron

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Fleming,R.A.; Olsen,D.J.; Savage,P.D.; Fox,J.L. Stability of ondansetron hydrochloride and cyclophosphamide in injectable solutions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 514–516.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.75
Injection volume: 20
Detector: UV 198

CHROMATOGRAM
Retention time: 2.98

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Add solid NaCl to a 500 μL aliquot of the reaction mixture until some solid remains undissolved, add 250 μL MeCN, stir for 5 min, inject a 20 μL aliquot of the upper layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:20 mM pH 4.4 KH_2PO_4 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 190

CHROMATOGRAM
Retention time: 16.3
Limit of detection: 18000 ng/mL

OTHER SUBSTANCES
Simultaneous: ifosfamide

REFERENCE
Lunn,G.; Sansone,E.B.; Andrews,A.W.; Hellwig,L.C. Degradation and disposal of some antineoplastic drugs, *J.Pharm.Sci.*, **1989**, *78*, 652–659.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:30 mM sodium dihydrogen phosphate 23:77
Flow rate: 1
Injection volume: 20
Detector: UV 195

CHROMATOGRAM
Retention time: 6.8

OTHER SUBSTANCES
Simultaneous: doxorubicin (5.4)

KEY WORDS
stability in 0.9% sodium chloride injection USP

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2375–2385.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 30:70
Flow rate: 1.5
Injection volume: 25
Detector: UV 200

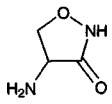
CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
buffer

REFERENCE
Kensler,T.T.; Behme,R.J.; Brooke,D. High-performance liquid chromatographic analysis of cyclophosphamide, *J.Pharm.Sci.*, **1979**, *68*, 172–174.

Cycloserine



Molecular formula: $C_3H_6N_2O_2$
Molecular weight: 102.09
CAS Registry No.: 68-41-7
Merck Index: 2820
Lednicer No.: 3 14

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 250 μL buffer + 30 μL 240 μg/mL 6-aminocaproic acid + 50 μL 6.5 mg/mL 5-methoxyindole-3-acetic acid, filter (Centriflo ultrafilter) while centrifuging at 723 g for 15 min, inject a 20-25 μL aliquot of the ultrafiltrate. Urine. 1 mL Urine + 30 μL 240 μg/mL 6-aminocaproic acid + 50 μL 3 mg/mL α-aminobutylhistidine + 1 mL 200 mg/mL sodium carbonate in water (prepare only 5-6 samples at a time), mix, add 2 mL isopropanol, vortex, centrifuge at 723 g for 5 min, inject a 25 μL aliquot of the top organic layer. (Buffer was 12.4 g boric acid + 100 mL 1 M NaOH diluted to 250 mL with water, pH 9.75.)

HPLC VARIABLES

Guard column: 30-38 μm Co:Pell ODS

Column: 240 × 5 10 μm ODS-Hypersil

Mobile phase: Isopropanol:water:glacial acetic acid:decanesulfonate 75:800:5:0.5 (plasma) or 65:800:5:0.5 (urine), pH adjusted to 4.4 with 1 M KOH

Flow rate: 2.3

Injection volume: 20-25

Detector: F ex 340 em 455 following post-column derivatization. Reagent at 1.2 mL/min is mixed with column effluent, mixture flows through a 250 × 4.5 reactor packed with 50 μm glass beads to the detector. (Reagent was o-phthalaldehyde and 2-mercaptoethanol post-column derivatizing reagent (Fluoraldehyde, Pierce).)

CHROMATOGRAM

Retention time: 5 (plasma), 6 (urine)

Internal standard: 6-aminocaproic acid (9.5), 5-methoxyindole-3-acetic acid (11 (plasma), 8 (urine), detection at UV 313), α-aminobutylhistidine (15)

Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES

Extracted: acetylacetonylcycloserine, metabolites

KEY WORDS

plasma; methoxyindoleacetic acid is IS for acetylacetonylcycloserine (UV 313 detection for both); post-column reaction

REFERENCE

Musson,D.G.; Maglietto,S.M.; Hwang,S.S.; Gravellese,D.; Bayne,W.F. Simultaneous quantification of cycloserine and its prodrug acetylacetonylcycloserine in plasma and urine by high-performance liquid chromatography using ultraviolet absorbance and fluorescence after post-column derivatization, *J.Chromatogr.*, **1987**, *414*, 121–129.

SAMPLE

Matrix: urine

Sample preparation: Filter (0.45 μm) urine. Remove a 1 mL aliquot and add a 20-fold molar excess of 2 mM 9-chloro-10-methylacridinium triflate in MeCN:pH 5.0 phosphate buffer 50:50, vortex for 10 s, heat at 70° for 30 min, add 1 mL glacial acetic acid, mix,

inject a 10 μL aliquot. (Synthesis of 9-chloro-10-methylacridinium triflate is as follows. Dissolve 6.07 g 9-chloroacridine (Eastman) in 55 mL dry dichloromethane, add 5 g methyl trifluoromethanesulfonate, stir for 3 h, filter, wash the solid with cold dichloromethane, dry in air overnight, recrystallize from MeCN to obtain 9-chloro-10-methylacridinium triflate as yellow crystals (mp 227-229°).)

HPLC VARIABLES

Column: 250 × 4.6 Partisil silica
Mobile phase: MeCN:EtOH:glacial acetic acid 50:30:20
Flow rate: 2
Injection volume: 10
Detector: F ex 257 em 475

CHROMATOGRAM

Retention time: 5.3
Limit of quantitation: 150 ng/mL

KEY WORDS

derivatization

REFERENCE

Yoo,G.S.; Choi,K.; Stewart,J.T. Second derivative ultraviolet spectrophotometry and high performance liquid chromatography with fluorometric detection of cycloserine using 9-chloro-10-methylacridinium triflate as a new UV and fluorescent labeling agent, *Anal.Lett.*, **1990**, *23*, 1245–1263.